## REMARKS

With the foregoing amendments, claims 9-25, 36-38, 40, 49-58 and 60 are presented for favorable consideration. Claims 39 and 59 have been cancelled and the subject matter of those claims has been added to claims 36-38 and 54-58. No new matter has been added by the claim amendments.

At the outset, applicants note with appreciation the Examiner's statement on page 3 of the Office Action that claims 9-25, 51-53 and 56-59 contain allowable subject matter.

Turning to the rejections, claims 36-50, 54-55 and 60 stand rejected under 35 USC 102(e) as allegedly being anticipated by Gainer 6,060,511 (the '511 patent). Applicants believe the rejection may have meant to refer to Section 102(b) because of its date of issuance, and, therefore, addresses the rejection under this portion of the statute for the Examiner's convenience. In any event, applicants respectfully request the withdrawal of the rejection for at least the following reasons.

Amended claim 36 relates to the use of TSC of a specific purity (i.e., the ratio of absorbencies is "**greater than 7.5**") for increasing the oxygen diffusivity. The '511 patent does not disclose at least this claim element. The '511 patent merely discloses that TSC should be "substantially pure" in order to increase the diffusivity. Significantly, the TSC material disclosed in the '511 patent was prepared from crocetin. This TSC material is not the highly purified material of the subject invention, and the subject application is not attempting to claim or cover the TSC material disclosed in the '511 patent. Moreover, there is no disclosure in the '511 patent of the use of the claimed highly purified synthetic material of the subject invention for increasing oxygen diffusivity.

In further support of the patentability of the claimed invention, and as noted on page 7 of the subject application, performing the Craw and Lambert analysis on the TSC disclosed in the '511 patent (i.e., TSC made by reacting naturally occurring saffron with sodium hydroxide followed by extractions which select primarily for the trans isomer), the purity value obtained averages about 6.8. In contrast, the purity level of the synthetic material of the claimed invention is significantly and unexpectedly higher. In this regard, the Craw and Lambert 1983 article relating to crocetin (submitted with the IDS of June 30, 2004), they note at page 241, "The crude sample gave a ratio of **3.1** which increased to **6.6 after purification**." Thus, the claimed invention is not anticipated by the '511 patent.

Similarly, Claim 37 relates to the use of TSC of a specific purity for treating emphysema (i.e., a condition which is characterized by a decrease in oxygen consumption). Claim 37 requires a specific purity, i.e., a ratio "greater than 7.5," for the usage of a BTCS for emphysema. There is no disclosure in the '511 patent of the use of the claimed highly purified material for treating emphysema.

Similarly, Claim 38 relates to the use of TSC of a specific purity for treating hemorrhagic shock. Claim 38 requires a specific purity (i.e., a ratio "greater than 7.5") on the usage of a BTCS for hemorrhagic shock. There is no disclosure in the '511 patent of the use of the claimed highly purified material for treating hemorrhagic shock.

Claim 40 relates to the administration of TSC via inhalation. Inhalation of TSC is not disclosed in the '511 patent (and it is not obvious that it could be given via inhalation).

Although the rejection identifies Claims 41-48, they have already been cancelled due to a restriction requirement.

Claim 49 relates to the use of a BTCS that is "not TSC" for the treatment of ischemia. The '511 patent simply discusses using crocetin for treating myocardial infarction. There is no discussion in the '511 patent of the use of a BTCS that is not TSC for the treatment of ischemia.

Claim 50 relates to the treatment of traumatic brain injury with a BTCS that is not TSC. Traumatic brain injury is not discussed in the '511 patent. Further, the use of a BTCS that is not TSC for treating traumatic brain injury is not discussed in the '511 patent.

Like Claims 36-38, Claim 54 relates to the treatment of ischemia with TSC of a specific purity, i.e., the ratio of absorbencies is "**greater than 7.5.**" There is no disclosure in the '511 patent of the use of the claimed highly purified material for treating ischemia.

Similarly, Claim 55 relates to the treatment of traumatic brain injury with TSC of a specific purity, i.e., the ratio of absorbencies is "greater than 7.5." There is no disclosure in the '511 patent of the use of the claimed highly purified TSC material for treatment of traumatic brain injury.

Finally, Claim 60 relates to treating, preventing or reducing the amount of ischemia resulting from surgery by administering a BTCS before, during or after surgery. This claim relates specifically to ischemia that can result from surgery. The '511 patent does not disclose such a use.

For at least the foregoing reasons, applicants request the withdrawal of the rejection based on the '511 patent.

Claim 60 also stands rejected under 35USC 112, first paragraph, because the specification allegedly does not provide enablement for the prevention of ischemia. The Office Action states that while "applicants have shown broadly that the instant compounds treat ailment by increasing the amount of oxygen available to the body, there is no correlation that these

compounds when given can prevent mammals from getting the disease." Applicants respectfully request the withdrawal of this rejection for the following reasons and based on the attached documentation.

Claim 60 relates to a method of treating, preventing or reducing the amount of ischemia resulting from surgery of a mammal by administering to the mammal before, during or after surgery a therapeutically effective amount of a BTSC. The application teaches one of ordinary skill in the art how to use the compounds of the subject invention to prevent ischemia (insufficient blood flow to tissues or organs) that can be associated with surgery, i.e. by administration of an effective amount prior to surgery. As specifically stated in the application, "Bipolar trans carotenoid salts are beneficial as a pretreatment for surgery, or as a treatment during or after surgery." *See* page 18, lines 3-4, of the application and other sections of the application that describe the usage of the invention.

Moreover, this important invention is confirmed by the attached article, which notes: "*Preconditioning with TSC substantially and significantly reduced the volume of cerebral infarction.*" *See* last sentence of Summary section of attached document. Applicants will be submitting a declaration in further support of this information and the claimed invention.

All of this information confirms that the application complies with each aspect of Section 112, and that the applicants have invented the important subject matter covered by Claim 60.

In view of the foregoing amendments and remarks, and the attached documentation, applicants submit that this application is in allowable condition. A notice to that effect is earnestly solicited.

If the examiner has any questions concerning this case, the undersigned may be contacted at 703-816-4009.

Respectfully submitted,

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# Ischemic tolerance induced by Trans-Sodium Crocetinate in Focal Cerebral Ischemic model

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*Summary*

***Trans-sodium crocetinate (0.125 mg/kg total dosage) or vehicle was administered intravenously as follows 1) 0.1 ml injection at 10 minutes after the onset of ischemia, 2) continuous infusion at a rate of 0.01 ml/min for the next 60 minutes, and 3) a final 0.1 ml injection 30 minutes after the cessation of continuous infusion. Twenty-four hours later, the animals were subjected to a transient period of ischemia by occluding simultaneously both common carotid arteries and the left middle cerebral artery. Infarction volumes were measured 24 hours after the ischemic event. Preconditioning with TSC substantially and significantly reduced the volume of cerebral infarction.***

*General procedures*

*Drug Administration:*

The effects of TSC on focal ischemic injury were examined using adult male Sprague-Dawley rats, each of which weighed between 330 and 370g. All procedures were approved by the University of Virginia Animal Care Committee. The animals were initially anesthetized in a chamber with a mixture of 4% halothane in oxygen gases. After being completely anesthetized, orotracheal intubation was performed and orotracheal tube was connected to a ventilator (Rodent Ventilator model 638, Harvard Apparatus, Holliston, MA). The right femoral artery and vein was cannulated for blood pressure monitoring, repeated blood gas analysis (348 Blood Gas Analyzer, Bayer HealthCare, Tarrytown, NY) and infusion of drug. Rectal temperature was monitored continuously

and maintained at 37°C with a heating lamp. During the operation, the concentration of halothane was controlled around 1.5% in $N_2$:$O_2$ (50%:50%) to keep the mean arterial blood pressure around 110mmHg.

*Three Vessel Occlusion Model:*

Focal ischemia was performed by clipping(Sundt AVM microclip No1, Codman & Shurtleff, Inc., Raynham, MA) the MCA at a point distal to the origin of the lenticulostriate arteries. The polypropylene suture loops around both CCAs were closed at the same time. Loss of blood flow was confirmed visually. The microclip on MCA and polypropylene snares around the both CCAs were removed 120 minutes after ischemic onset and recirculation was confirmed by observing directly. After 24 hours, infarct volume was calculated.

24 hours after ischemic onset, animals were anesthetized with an over dose of pentobarbital and killed by decapitation. The brains were removed rapidly and 2mm thickness of coronal sections were cut with a McIlwain tissue chopper and stained in 2% 2,3,5 triphenyltetrazorium chloride (TTC) in phosphate-buffered saline for 5min at 37°C and then placed in 10% paraformaldehyde solution.

Infarction volume was calculated by summing the infarction areas in individual sections using image analysis software (Scion Image Beta 4.02, Scion Corporation, Frederick, MD) . In addition, the areas of the hemispheres ipsilateral and contralateral to the occluded MCA were measured, and the total volume of each hemisphere was calculated in similar manner. The actual infarct volume, adjusted for swelling, was calculated using the following formula: total infarct volume x (contralateral hemisphere

volume/ipsilateral hemisphere volume). The values shown in both text and figures are the calculated actual infarct volume.

**Results**

Three-vessel occlusion elicited cerebral infarction volumes in the 3VO-only and 3VO+Vehicle groups of 165.6 $\pm$ 47.2 $mm^3$ and 133.9 $\pm$ 17.5 $mm^3$ (mean $\pm$ SEM), respectively. The infarct volumes in these two groups did not differ significantly (one-way ANOVA). Trans-sodium crocetinate reduced infarct volume substantially. Infarct volume in the 3VO+TSC group was 57.7 $\pm$ 16.2 $mm^3$ . This decrease in infarct volume achieved statistical significance when the 3VO+TSC group was compared to either the 3VO-only or 3VO+Vehicle group ($p < 0.01$, one-way ANOVA with the Holm-Sidak *post hoc* test).

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